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RESEARCH ARTICLE

Changes in trophic characteristics of two fish species of *Astyanax* (Teleostei: Characidae) in response to aquatic pollution

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ABSTRACT. The trophic plasticity of most fish species of *Astyanax* Baird & Girard, 1854 in response to environmental changes and resource availability is high. This work evaluates the differences in the trophic characteristics of two congeneric species, *Astyanax taeniatus* (Jenyns, 1842) and *Astyanax lacustris* (Lütken, 1875), in Rio das Velhas Basin, which is highly impacted by the discharge of sewage from the Metropolitan Region of Belo Horizonte (MRBH). Eight sites were sampled and grouped into three regions: upper course (two sites upstream of the MRBH); middle course (three sites located in the middle portion of the Rio das Velhas, region with greater influence of the MRBH), and lower course (three sites downstream of the MRBH). Samples of fish and food resources were collected from all sites to obtain the isotopic composition of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$), and the stomach contents of the two species was analized. The most common items in the stomach of *A. lacustris* and *A. taeniatus*, respectively, were from plants and insects, followed by algae/periphyton (especially at the low course of Rio das Velhas). In contrast, stable isotope analyses indicated that algae (in polluted sites) and periphyton (in least-disturbed sites) were best assimilated both species. Both analyses indicated that the trophic niches of the two species overlap more in more polluted sites relative to less polluted sites. *Astyanax taeniatus* and *A. lacustris* only presented different isotopic composition of carbon and nitrogen in the upper course of the Rio das Velhas, probably in response to the greater diversity of food items consumed by each species. In the other regions, the species presented similar isotopic signatures, with $\delta^{15}N$ and $\delta^{13}C$ notably enriched in the most polluted regions (middle and low course). Our results suggest that pollution acts by increasing trophic niche overlap of these species, altering the type of resources most assimilated, and promoting a greater enrichment of $\delta^{15}N$ in fish and resources.

KEY WORDS. *Astyanax lacustris*, *Astyanax taeniatus*; carbon, nitrogen enrichment, stable isotopes, stomach contents.

INTRODUCTION

In many developing countries, a large proportion of untreated raw sewage is released into aquatic environments (Macedo and Sipaúba-Tavares 2010), increasing the load of organic matter and pollutants in rivers, which are considered as the main drivers of artificial eutrophication in these environments (Tundisi and Tundisi 2008). Nutrients from human activities, when released into the water, contribute to the rapid growth of algal blooms and aquatic plants, altering the physico-chemical and ecological conditions of aquatic systems (Pereira and Mercante 2005, Hicks et al. 2016, Baeta et al. 2017). Among the consequences of an increase in primary productivity is the rapid reduction in water oxygen levels, which has drastic effects on fish and invertebrate communities (Macedo and Sipaúba-Tavares 2010, Baeta et al. 2017). In addition, changes in primary productivity in response to pollutants affect directly the diets of these aquatic consumers (Cabana and Rasmussen 1996, Esteves and Aranha 1999), and may also be responsible for promoting the local extinction of specialist and less tolerant species.

By favoring primary productivity, environmental pollution of aquatic systems may homogenize the type of resources available to organisms in higher trophic levels. This process of homogenization in aquatic systems has been also described in several taxonomic groups such as diatoms, zooplankton and macroinvertebrates (Lougheed et al. 2008). Such changes in the balance of available resources may consequently affect the food web since changes in nutritional composition or abundances of basal food sources can induce shifts in primary consumers or even their exclusion (Hall 2004). However, the effects of this homogenization of producer communities on upper trophic levels remains unclear.

According to ecological theory, generalist species are less sensitive to environmental change than specialists as they have the capacity of varing their diet according to the availability of resources present in their respective habitats (Tundisi and Tundisi 2008). Therefore, trophic plasticity is an important strategy to allow species to tolerate changes in environment condition (Gerking 1994, Wootton 1999). Species of the American fish *Astyanax* Baird & Girard, 1854 are well known for their broad geographic distribution and their ability to inhabit environments with differing levels of preservation, including highly polluted environments (eg., Souza and Lima-Júnior 2013, Carvalho et al. 2015). *Astyanax* is composed of approximately 100 species that are distributed from the southern United States to northern Argentina (Eigenmann 1921, Géry 1997, Weitzman and Fink 1983). Most species have omnivorous feeding habits, with diets composed of animal and vegetable items, of both autochthonous and allochthonous origins (e.g., Esteves 1996, Vilela et al. 2002, Cassemiro et al. 2002, Bennemann et al. 2005). In addition, some species of this genus present generalist feeding habits and high trophic plasticity in response to environmental changes and resource availability (Lobón-Cerviá and Bennemann 2000, Carvalho et al. 2015), which increases their chance of survival in disturbed habitats (Menezes et al. 2007). However, congeneric species (species of the same genus) may respond differently to changes in the aquatic environment and the availability of resources.

One way to identify how distinct species respond to changes in the environment is by comparing their feeding habits in regions under differing levels of human disturbance (e.g, Carvalho et al. 2015). Accordingly, analyses of stomach contents and stable isotopes (carbon and nitrogen), can be used simultaneously for robust and reliable assessment of feeding habits (e.g., Carassou et al. 2017, Connan 2017). Stomach contents analyses provide useful taxonomic information on consumed prey items (Beaudoin et al. 1999). However, there are often uncertainties in the identification of such items, due to the different stages of digestion of food items, and that not all ingested items are in fact assimilated into biomass (Manetta and Benedito-Cecílio 2003). Stable isotopes analyses, on the other hand, provide information on the energy flow in food chains (Peterson and Fry 1987, Kling et al. 1992). The nitrogen ($\delta^{15}N$) isotope is consistently fractionated throughout the trophic web, allowing researchers to make inferences about the trophic relationships of consumers with their diet (Vander Zanden et al. 1997, Post 2002, Vanderklift and Ponsard 2003). The carbon ($\delta^{13}C$) isotope, in turn, allows to delineate the energy flow in environments that present several foods with different carbon values (Manetta and Benedito-Cecílio 2003).

Based on this information, we aimed to evaluate how trophic characteristics of two congeneric species, *Astyanax lacustris* (Lütken, 1875) and *Astyanax taeniatus* (Jenyns, 1842), change across an environmental pollution gradient. The diet and the trophic niches occupied by these two species were evaluated in different regions of a highly disturbed Neotropical river basin, the Rio das Velhas, south east Brazil. The main source of disturbance in this river basin is the discharge of untreated domestic and industrial sewage from a large nearby urban conurbation. We tested the following hypotheses: 1) Under natural (undisturbed) conditions, the congeneric species occupy different trophic niches, and consequently present little food overlap; 2) However, along a gradient of pollution, due to the simplification (homogenization) of the available resources, and due to their high trophic plasticity, both species will increase their food overlap and will present more similar isotopic signatures.

MATERIAL AND METHODS

The study was conducted in the Rio das Velhas Basin, southeast Brazil, with sampling sites located in the main channel of the Rio das Velhas. The Rio das Velhas is the largest tributary of the São Francisco river Basin (Alves and Pompeu 2001), and is located entirely in the territory of Minas Gerais state (CETEC 1983), covering 51 municipalities. The basin, with a drainage area of 29,173 km^2, has an average annual flow rate at its mouth of 300 m^3/s and average width of 38.3m (CETEC 1983). The Rio das Velhas is of significant economic and social importance. Its upper course is located at the Metropolitan Region of Belo Horizonte (MRBH), the third largest urban conurbation in Brazil, with almost six million inhabitants, and is the main water supply.

Eight sites were sampled along the Rio das Velhas channel (RV-01 to RV-08), which were divided into three regions (upper, middle and lower course). The upper course of the Rio das Velhas (Upper RV) corresponds to the region with the best water quality (RV-01 and RV-02). The middle course (Middle RV) is in the region with the greatest influence of the MRBH, characterized by the discharge of large amounts of domestic and industrial sewage (RV-03, RV-04, and RV-05). The lower course (Low RV), in turn, is the most distant region from the MRBH and is close to the river mouth (RV-06, RV-07, and RV-08). In this region the river partly recovers its quality, due to the presence of numerous well preserved tributaries (Alves and Pompeu 2001) (Table 1, Fig. 1).

Two sewage treatment plants (STP), Arrudas and Onça, were also sampled to obtain complementary samples of the suspended material to obtain the isotopic composition of the raw

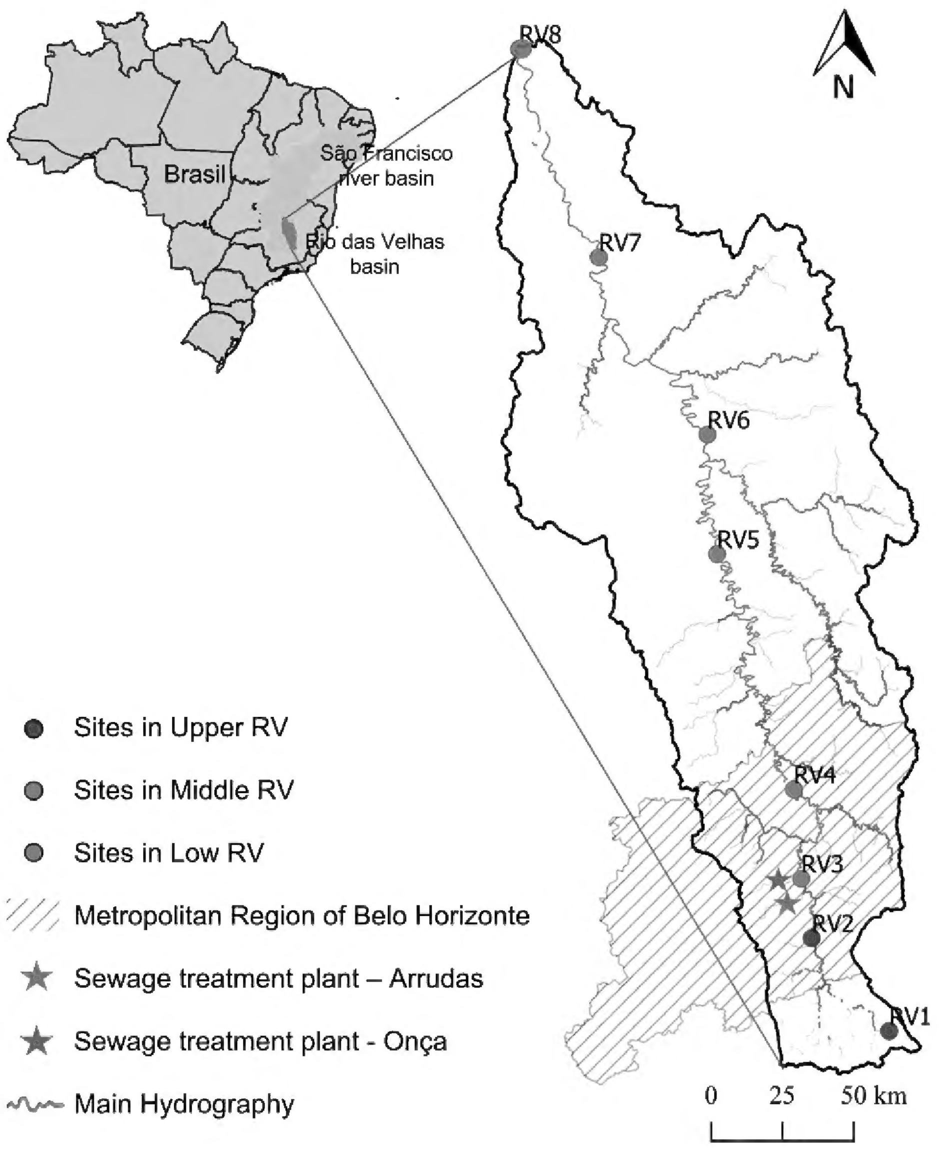


Figure 1. Sampling network in the Rio das Velhas Basin, Minas Gerais, Brasil.

sewage. All the sites were sampled between the years 2015 and 2016, in the dry (May to August) and wet (October to January) seasons (Table 1, Fig. 1).

The information about degradation level of sampling sites was obtained through data from literature (Feio et al. 2015). The sites RV08, RV11, RV10, RV12, RV13, RV14, RV15 and RV16 (Feio et al. 2015) were considered as the correspondents of RV-01, RV-02, RV-03, RV-04, RV-05, RV-06, RV-07 and RV-08, respectively (Table 1). Degradation levels range from I (preserved) to IV (degraded).

Data about water quality, hypereutrophic condition, toxic contamination and pressure factors in the study sites were accessed through IGAM's website (http://portalinfohidro.igam.mg.gov.br), which monitors water quality quarterly at several points across the Rio das Velhas Basin. The IGAM monitoring sites: BV001, BV139, BV105; BV137; BV141, BV150, BV151 and BV149, were considered as sampling points: RV-01, RV-02, RV-03, RV-04, RV-05, RV-06, RV-07 and RV-08, respectively (Table 1). Mean values of conductivity, dissolved oxygen, total ammoniacal nitrogen and total phosphorous were obtained from IGAM measurements carried out in the years 2015 and 2016. The hypereutrophic condition, toxic contamination and pressure factors acting in the study sites were extracted from the quarterly report of the year 2017.

Table 1. Geographic location (in degrees/minutes/seconds and UTM, date, altitude and municipality) and water quality of the sampling sites sampled in the main channel of Rio das Velhas. Cond.: Condutivity (µS/cm), D.O.: Dissolved oxygen (mg/l), Am. nitr.: Ammoniacal nitrogen (mg/l), Phosp.: Total phosphorus (mg/l), Tox. contam.: Toxic contamination, Deg. level: degradation level ranging from I to IV (Feio et al. 2015). *Sites with hypereutrophic condition according IGAM.

Characteristics of sampling sites						Water Quality						
Regions	Sampling points	Date of sampling	Coordinates	Altitude (m)	Municipality	Cond.	D.O.	Am. nitr.	Phosp.	Tox. contam.	Deg. level	Pressure factors
Upper RV	RV-01	20/08/2015 21/01/2016 10/06/2016	20°18'42.8"S 43°34'01.5"W 23K 649606 E 7753356 W	1010	Ouro Preto	26.09	8.10	0.11	0.06		I	
Upper RV	RV-02	19/08/2015 20/01/2016 9/06/2016	20°01'10.7"S 43°49'45.4"W 23K 622454 E 7785916 W	729	Nova Lima	73.21	7.54	0.12	0.08		II	
Middle RV	RV-03	17/08/2015 19/01/2016 7/06/2016	19°49'54.8"S 43°51'56.2"W 23K 618796 E 7806723 W	674	Santa Luzia	345.15	2.95	5.40	0.69	Total ammoniacal nitrogen	III	Discharge of domestic sewage
Middle RV	RV-04	18/08/2015 18/01/2016 8/06/2016	19°32'56.7"S 43°53'33.3"W 23K 616174 E 7838041 W	658	Lagoa Santa	330.92	4.29	4.96	0.49	Total ammoniacal nitrogen	III	Discharge of domestic sewage
Middle RV	RV-05	10/08/2015 11/01/2016 31/05/2016	18°48'19.2"S 44°09'09.2"W 23K 589298 E 7920498 W	567	Curvelo	287.20	7.25	0.92	0.41	Arsenic and total ammoniacal nitrogen	III*	Gold metallurgy and discharge of domestic sewage
Low RV	RV-06	11/08/2015 12/01/2016 1/06/2016	18°25'33.2"S 44°11'10.9"W 23K 585926 E 7962502 W	552	Corinto	203.23	7.30	0.23	0.22	Arsenic	II	Agriculture
Low RV	RV-07	13/08/2015 13/01/2016 3/06/2016	17°51'55.4"S 44°32'57.4"W 23 K 547752 E 8024649 W	495	Lassance	162.21	7.63	0.21	0.17	Arsenic	II	Discharge of domestic sewage and agriculture (sugar cane)
Low RV	RV-08	12/08/2015 14/01/2016 2/06/2016	17°12'25.9"S 44°48'49.8"W 23 K 519793 E 8097515 W	464	Várzea da Palma	153.00	8.35	0.14	0.11	Arsenic	II	Discharge of domestic sewage
Sewage MRBH	STP Arrudas	20/07/2016, 25/01/2017			Sabará							
Sewage MRBH	STP Onça	20/07/2016, 18/01/2017			Belo Horizonte							

Captures of specimens of *A. lacustris* and *A. taeniatus* were carried out with gillnets with mesh sizes of 2.4, 3.0 and 4.0 cm between opposing nodes and with cast nets, seines and sieves. A total of 137 individuals of *A. lacustris* and 103 individuals of *A. taeniatus* was sampled in the three regions. The captures with gillnets represented 63% of sampling. For the stable isotope analyses, we collected at least five samples of each species at each sampling site (whenever possible). In the field, dorsal muscle was removed for large specimens and for small the whole fish was used removing the digestive tract. All samples were kept frozen until laboratory processing to avoid decomposition and deterioration of the material. In the laboratory, the fish samples were lyophilized for 24 hours, ground to fine and homogeneous powder using mortar and pestle and stored in eppendorf tubes.

The individuals that were not selected to stable isotopes analyses were fixed in formalin (10%) in the field, washed in water after fixation and transferred to alcohol (70%) in laboratory. Individuals predated or in high stage of decomposition were discarded. The remain individuals were used to stomach contents analyses in laboratory, where they had their stomach contents carefully removed. The same individuals were not used for both isotopic and stomach contents analyses because the stomach contents were analyzed following the results of stable isotope analyses, when we detect the need for complementary information.

Whenever possible, we collected five samples of all basal food resources available at each sampling site: periphyton, filamentous algae, suspended matter, fine particulate organic matter (FPOM) from sediments, vegetation (grasses and riparian vegetation), coarse particulate organic matter (CPOM), and aquatic macrophytes. Complementary samples of the suspended material were made at the sewage treatment plants to obtain the isotope signature of the raw sewage.

Samples of algae, aquatic macrophytes, vegetation and CPOM were collected at all sites where they were present, stored in plastic bottles and kept frozen until laboratory processing. Filamentous algae and aquatic macrophytes were collected manually in each site where they were present. Leaves from pasture (grasses) and from the natural riparian vegetation were manually collected along river banks in each site, with the most common species being prioritized at the site. The CPOM was randomly collected from leaf litter deposits in the streams.

Liquid samples, like periphyton, suspended matter (including sewage samples) and sediment, were collected at each site and kept frozen until processing in laboratory, where they were filtered using a filtration device attached to a vacuum pump using calcined quartz fiber filters (Whatman® QMA quartz filters). The periphyton was collected by scraping rocks with a brush and placing the material in a plastic bottle with distilled water. FPOM samples were collected from sediment deposits revolving in each sampling site and stored in plastic bottles. The suspended matter presented in the sampling sites and at STPs were collected with a phytoplankton net (0.45 mm mesh) deployed for a period of three minutes at each sampling site.

In the laboratory, all basal resource samples were dried in an oven at 60 °C for 48 hours and then ground with a mortar and pestle and stored in Eppendorf tubes.

The contents of 44 stomachs of *A. lacustris*, and 31 stomachs of *A. taeniatus* were analyzed in total. Food items were weighed (0.001 g accuracy/ wet weight) and identified under stereomicroscope to the lowest taxonomic category possible. The frequency of occurrence (Fi = number of times item i occurred, divided by the total number of stomachs) and the relative weight (Pi = sum of the weight of item i divided by the sum of the weight of all items) of each item were obtained. The food index (IA), proposed by Kawakami and Vazzoler (1980), was then calculated for each species and region, according to the formula: $IAi = (Fi.Pi)/\Sigma Fi.Pi$, where, IAi = food index of item i; Fi = frequency of occurrence of item i, and Pi = weight of item i.

The degree of overlap in food items between species was calculated using the simplified Morisita index (Morisita-Horn index) (Krebs 2014), according to the formula below: $C_H = 2\Sigma Pij.Pik/\Sigma P^2ij + \Sigma P^2ik$, where, C_H = Simplified Morisita Index of overlap (Horn 1966) between species j and species k; Pij = Proportion resource i is of the total resources used by species j; Pik = Proportion resource i is of the total resources used by species k, and n = Total number of resource states (I = 1, 2, 3, ... n).

For the food items characterization, "detritus" was considered dead particulate organic material, "sediment" included inorganic particles, and "plant remnants" were related to fragment of terrestrial vegetation.

A total of 42 samples of *A. lacustris*, 47 samples of *A. taeniatus* and 703 basal resources samples were sent to the Center for Nuclear Energy in Agriculture (CENA) at University of São Paulo (USP) for isotopic analysis. About 2–5 mg of dry animal tissue material and approximately 5–10 mg of basal resources samples were selected for analysis.

To determine the isotopic ratio, a mass spectrometer system in the Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as relative difference of international reference standards, in the delta notation (δ ‰), and calculated using the following formula: $\delta X = [(R_{sample}/R_{standard})^{-1}] \times 10^3$, where X is ^{13}C or ^{15}N and R represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie and Prosser 1996).

Differences in isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ of consumers and resources between the three regions were tested using one-way analysis of variance (ANOVAs) when the normality and homoscedasticity assumptions were met. The nonparametric Kruskal-Wallis test was used for data with non-normal distribution. When significant differences ($p < 0.05$) were observed, means were compared using the post-hoc Tukey's test. We also tested if isotopic signatures of *A. lacustris* and *A. taeniatus* presented variation between the dry and wet season, using t-tests (normal distribution) and Mann-Whitney tests (non-parametric). These analyses were performed in the software Statistica 6.0 (Statsoft 2004).

To evaluate the trophic structure of *A. lacustris* and *A. taeniatus* populations, individuals of the two species were plotted in the bi-plot space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis) in each region (Fig. S1). Source contributions to the *A. lacustris* and *A. taeniatus* diet were estimated for the three regions based on stable isotope data analyzed through Bayesian stable isotope mixed models (Moore and Semmens 2008, Parnell et al. 2010), specifically using the MixSIAR package in R (Stock and Semmens 2016a). For both analyses, only the autochtonous sources, algae and periphyton, and the allochtounous sources, leaves of riparian vegetation and grasses were taken into account. The samples of sewage were also considered to sites "Middle RV" and "Low RV" since they are located in the area under influence of pollution. We used Markov chain Monte Carlo sampling based on the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 and model 4 (Resid*Process) error structure (Stock and Semmens 2016b). Diagnostic tests (Gelmin-Rubin, Heidelberger-Welch and Geweke) and trace plots were examined for model convergence. The fractionation values used for consumers were 0.4 ± 1.3 ‰ for Carbon and 2.54 ± 1.27‰ for Nitrogen (Vanderklift and Ponsard 2003, Post 2002). Both the graphical representation and the partition analysis were done using the MixSIAR package in the R programming environment (Stock and Semmens 2016a).

The isotopic niches of *A. lacustris* and *A. taeniatus* in both regions (Upper RV, Middle RV and Low RV) were quantified based on standard ellipse areas (SEA – expressed in ‰^{2}) through use of the Stable Isotope Bayesian Ellipses package in R (SIBER, Jackson et al. 2011). The standard ellipse area (SEA) represents the core isotopic niche space and it is a proxy of the richness and evenness of resources consumed by the population (Bearhop et al. 2004). All measures were "bootstrapped" (n = 10,000, indicated by the letter "b") to compare groups with different sample sizes. A small sample size correction (indicated by the subscript letter "c") was applied to SEA to increase the accuracy of the comparisons, enabling the comparison of niches of populations with different sample sizes (Jackson et al. 2011). The SEAc allows to calculate the degree of niche overlap (in percentage, where 100% indicates total overlap) and can be used as a quantitative measure of diet similarity among different species (Hill et al. 2015).

RESULTS

Stomach contents

Only two stomachs were found empty, both of *A. taeniatus* sampled in Upper RV. Plant and insect remnants were the predominant items in the stomachs of *A. lacustris* and *A. taeniatus*, respectively (Table 2). However, both species presented variations in the type and proportion of ingested food items in each study region. In Upper RV, *A. lacustris* feed more on plant remnants, aquatic insects and detritus, while *A. taeniatus* feed on sediments and insect remnants. In the middle RV, *A. lacustris* maintained its diet based on plant remnants, however there was an increase of insect remnants. In this region *A. taeniatus* feed on insect remnants and aquatic insects. In the low RV, the most consumed item by *A. lacustris* was algae/periphyton, a pattern also observed for *A. taeniatus*, albeit to a lesser extent (Table 2).

Variation in resources used by *A. lacustris* and *A. taeniatus* was reflected in the food overlap of the two species in each region. The lowest food overlap was observed in the Upper RV (0%), followed by middle RV (34%) and low RV(83%).

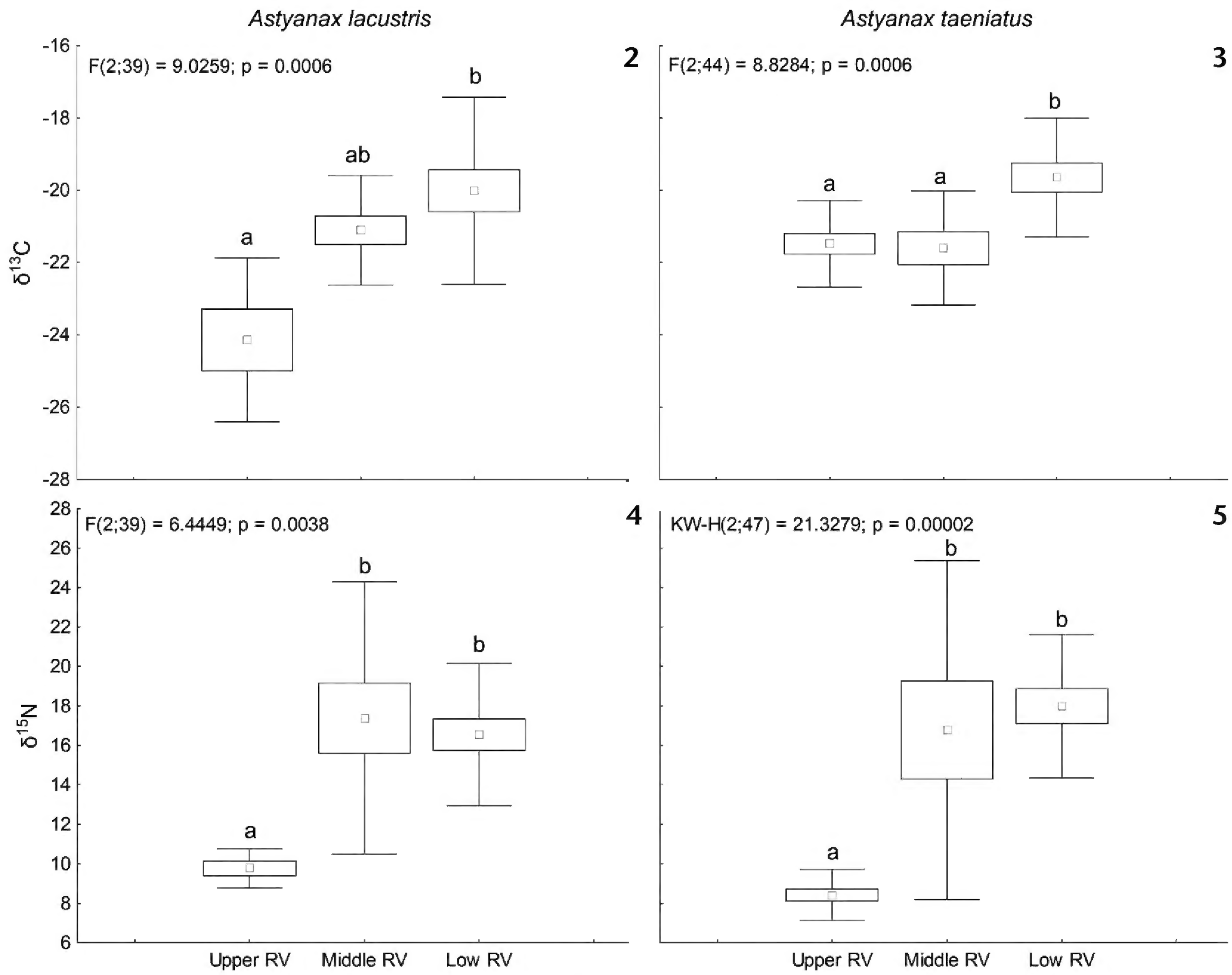
Table 2. Food index (AI), frequency of occurrence (Freq.) and weight of each food item found in the stomachs of the species *A. lacustris* and *A. taeniatus* in each sampled region of the Rio das Velhas Basin.

	Item	Upper RV			Middle RV			Low RV		
		IA	Freq.	Weight	IA	Freq.	Weight	IA	Freq.	Weight
Astyanax lacustris	Algae/ Periphyton	0.00	0.00	0.00	0.00	0.08	0.00	0.80	0.32	0.85
	Aq. Macrophytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01
	Sediment	0.00	0.00	0.00	0.00	0.15	0.00	0.02	0.24	0.03
	Detritus	0.20	0.17	0.33	0.00	0.00	0.00	0.00	0.00	0.00
	Plant remnants	0.40	0.33	0.33	0.53	0.54	0.44	0.00	0.16	0.00
	Aquatic insects	0.40	0.67	0.33	0.00	0.23	0.01	0.00	0.16	0.00
	Terrestrial insects	0.00	0.00	0.00	0.02	0.08	0.12	0.00	0.04	0.00
	Insects remnants	0.00	0.67	0.00	0.44	0.46	0.43	0.17	0.56	0.10
Astyanax taeniatus	Algae/ Periphyton	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.40	0.27
	Aq. Macrophytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Sediment	0.43	0.50	0.47	0.00	0.00	0.00	0.00	0.13	0.00
	Detritus	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.07	0.06
	Plant remnants	0.00	0.10	0.00	0.12	0.17	0.14	0.15	0.20	0.18
	Aquatic insects	0.00	0.20	0.00	0.64	0.17	0.77	0.00	0.00	0.00
	Terrestrial insects	0.00	0.00	0.00	0.02	0.17	0.03	0.10	0.07	0.34
	Insects remnants	0.57	0.60	0.52	0.22	0.67	0.07	0.28	0.40	0.16

Stable isotopes

The $\delta^{13}C$ and $\delta^{15}N$ of *A. lacustris* and *A. taeniatus* is different among the three regions (Figs 2–5). The $\delta^{13}C$ of *A. lacustris* is significantly different between Upper and Low RV (Fig. 2), while the $\delta^{13}C$ of *A. taeniatus* in Low RV is different of all other regions (Fig. 3). The $\delta^{15}N$ values of both species in Upper RV are different to the $\delta^{15}N$ values in Middle and Low RV (Figs 4, 5). When the comparison was made between the species, it was possible to observe that the isotopic composition of *A. lacustris* and *A. taeniatus* were different only in the upper RV for both carbon ($p < 0.01$) and nitrogen ($p = 0.02$). In other regions – middle RV ($\delta^{13}C$: $p = 0.41$ and $\delta^{15}N$: $p = 0.83$) and low RV ($\delta^{13}C$: $p = 0.61$ and $\delta^{15}N$: $p = 0.23$) – there was no variation between species. In addition, only the *A. taeniatus* presented variation in the $\delta^{15}N$ values between seasons ($p = 0.02$), with more enriched values being observed in the dry season.

Basal resources presented extensive variation in their isotopic composition, except for riparian vegetation and grasses, that did not vary in $\delta^{13}C$ between the three sampled regions (Table 3). Strikingly, autotrophic resources (algae, periphyton and aquatic



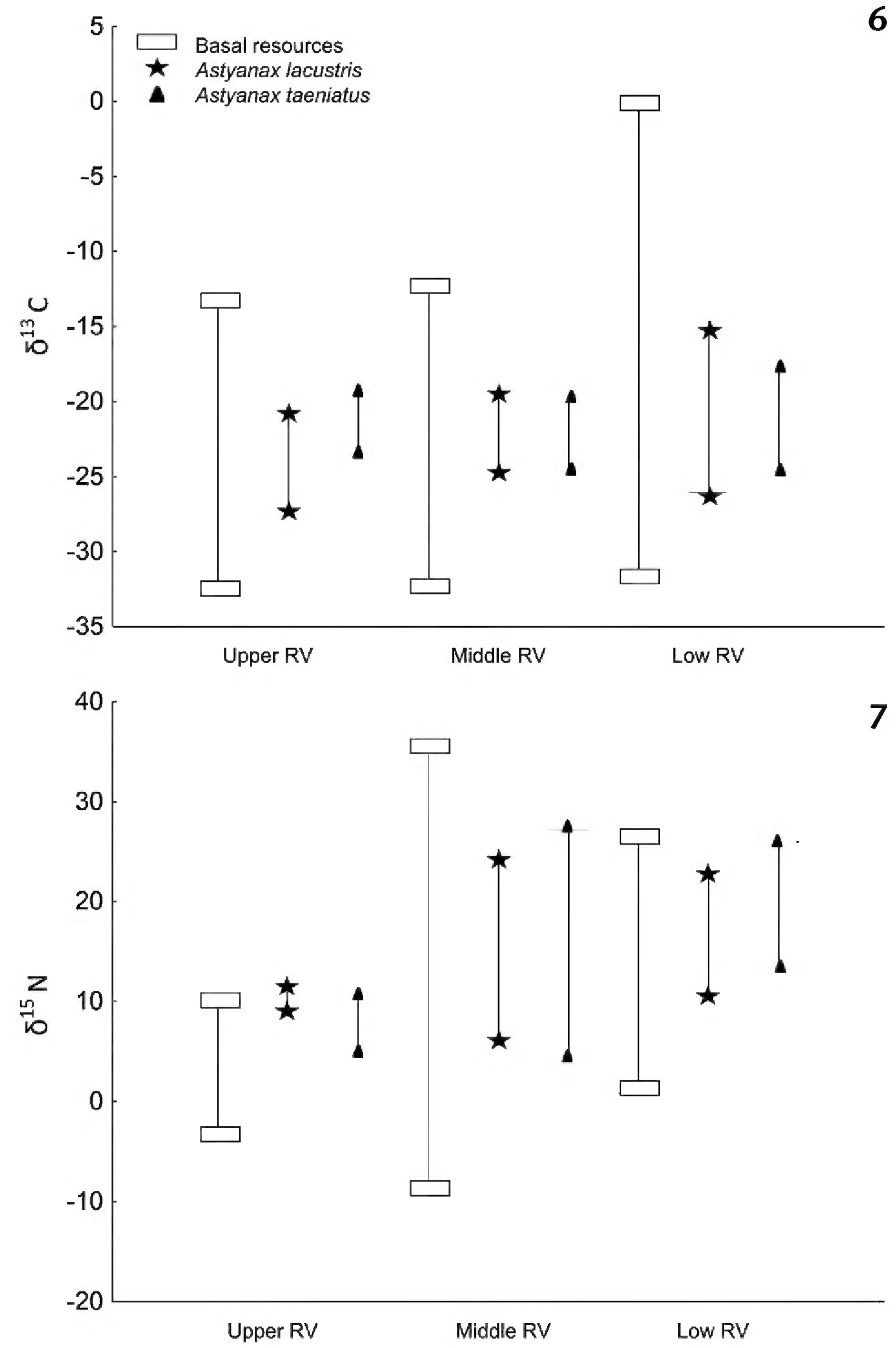
Figures 2–5. Variation in the isotopic composition of carbon (2–3) and nitrogen (4–5) in the species *A. lacustris* (2, 4) and *A. taeniatus* (3, 5) among the studied regions. Mean (small box), standard error (bars) and standard deviation (large box). Letters (a and b) indicate significant differences according to post-hoc Tukey's test.

Table 3. Variation in the carbon and nitrogen isotopic composition of the resources sampled in the three regions of the Rio das Velhas Basin. Letters (a, b and c) indicate significant differences according to post-hoc Tukey's test. AL: filamentous algae, CPOM: coarse particulate organic matter, FPOM: fine particulate organic matter, GR: grasses, MA: macrophytes, PE: periphyton, RV: riparian vegetation, SM: suspended matter and SW = raw sewage.

	$\delta^{13}C$ (mean and SD)				$\delta^{15}N$ (mean and SD)			
	Upper RV	Middle RV	Low RV	p	Upper RV	Middle RV	Low RV	p
AL	-27.74±4,66a	-22.84±6,35a	-5.52±2,47b	<0.01	4.67±2,99a	8.38±16,38ab	15.85±4,81b	<0.01
CPOM	-30.01±1,17a	-29.00±1,46b	-28.98±1,53b	<0.01	0.94±2,72a	4.48±3,86b	7.53±3,52c	<0.01
FPOM	-25.08±1,75a	-23.55±1,33b	-21.33±3,07c	<0.01	5.03±1,70a	6.66±7,10a	14.26±3,75b	<0.01
GR	-13.98±0,87a	-17.21±6,45a	-16.27±5,67a	0.56	0.28±1,81a	4.56±3,66b	8.00±3,89c	<0.01
MA	-30.54±1,08a	-25.94±5,91ac	-29.00±0,75c	<0.01	8.46±1,93a	19.55±14,18b	15.47±3,37b	<0.01
PE	-25.07±2,20a	-23.62±1,61a	-19.19±3,69b	<0.01	5.26±1,20a	9.15±10,72a	15.36±4,08b	<0.01
RV	-30.20±1,78a	-29.99±1,86a	-29.51±1,30a	0.54	0.64±1,72a	4.78±2,43b	7.28±2,39c	<0.01
SM	-25.88±1,67a	-24.56±1,75b	-20.45±4,62c	<0.01	4.47±1,39a	5.59±7,24a	14.15±4,96b	<0.01

macrophytes) showed highly enriched nitrogen isotopic values in the most polluted regions (middle and low RV) (Figs 6, 7). The range of carbon values of basal resources and fish species was higher in the low RV region and similar in other regions (Fig. 6). The range of nitrogen isotopic values, in turn, was higher in middle and low RV regions (Fig. 7).

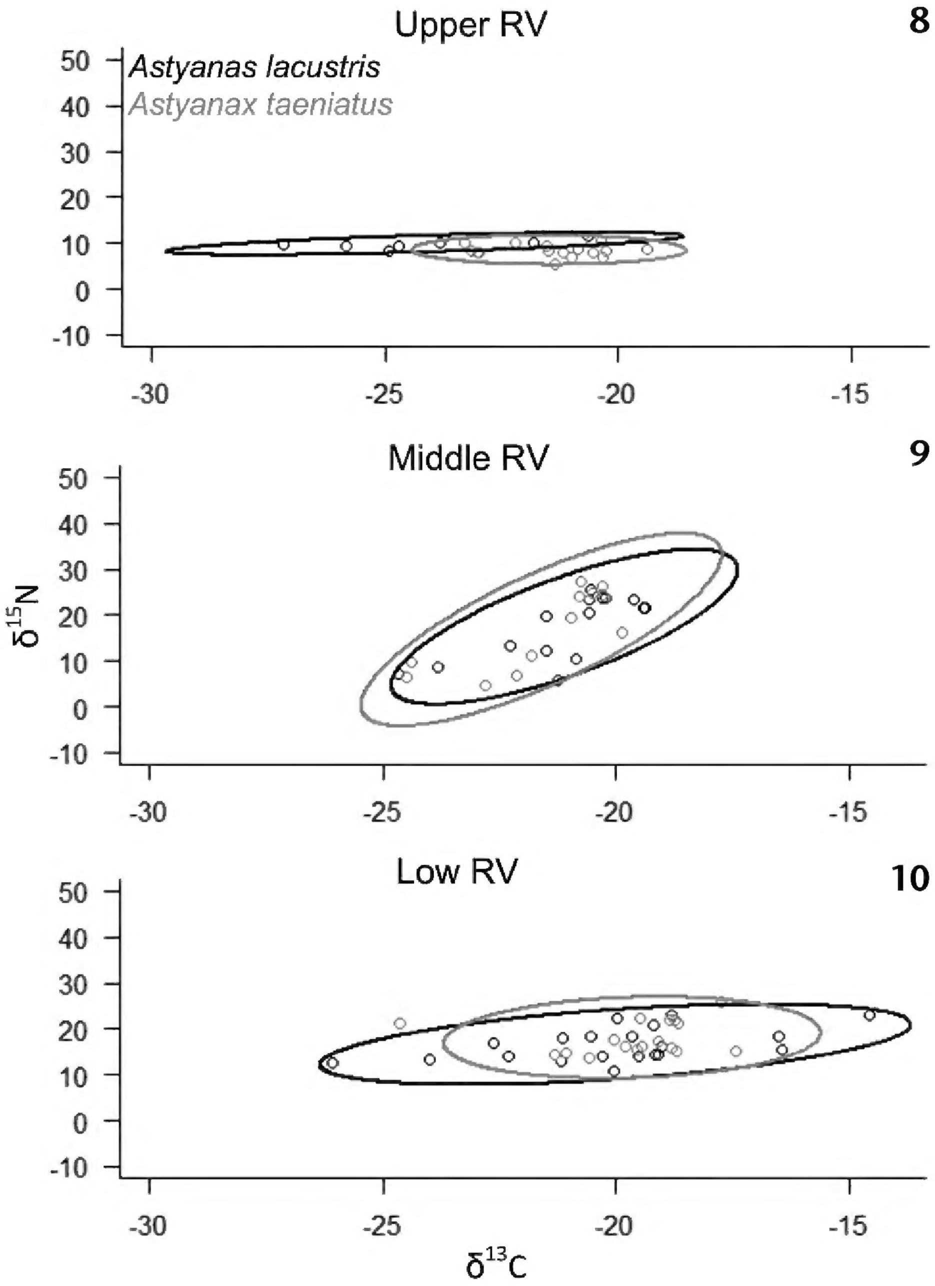
According to the partition analysis, in the upper RV, periphyton was the most assimilated basal resource, followed



Figures 6–7. Isotopic values range for $\delta^{13}C$ (6) and $\delta^{15}N$ (7) of basal resources, *A. lacustris* and *A. taeniatus* sampled in three regions of Rio das Velhas basin.

by filamentous algae and grasses (mainly for *A. taeniatus*). In the middle course, both species assimilated more carbon from filamentous algae and the other resources had similar contributions. In the lower course, the periphyton was again the most assimilated resource by *A. lacustris* and *A. taeniatus*. However, riparian vegetation had a greater contribution in this site than in other sites, being the second most consumed resource by both species (Table 4).

Results of isotopic niche overlap were similar to those observed in the stomach contents analyses. We again observed a slight overlap of trophic niches in the upper RV (23%) (Fig. 8, see also Fig. S1). In this region, with no influence of the sewage from the MRBH, the two species presented little overlap in assimilated carbon sources and appeared to occupy the same trophic level (Fig. 8). In the middle course of the Rio das Velhas, where the discharge of sewage is high, the carbon and nitrogen values of the two species were very similar, presenting high overlap (71%). In addition, a large variation in nitrogen signatures for both species was observed in this region, with an amplitude of 4.68 to 27.22 ‰ (Figs 7, 9). In the low RV it was also observed a high niche overlap (62%) especially in the carbon source (Fig. 10). However, there was a decrease in variation of nitrogen isotopic composition (10.97 to 25.96 ‰) (Fig. 7).



Figures 8–10. Trophic niche of *A. lacustris* and *A. taeniatus* (evaluated by the ellipse area with 95% confidence interval) in the Upper (8), Middle (9) and Low (10) regions of the Rio das Velhas Basin.

Table 4. Mean proportion of each basal resource assimilated by *Astyanax lacustris* and *A. taeniatus* at each sampling site. AL: filamentous algae, SW: raw sewage, GR: grasses, RV: riparian vegetation, PE: periphyton.

Rio das Velhas regions		Basal resources				
		AL	SW	GR	RV	PE
A. lacustris	Upper	0.31		0.06	0.04	0.59
	Middle	0.58	0.09	0.08	0.08	0.18
	Low	0.04	0.04	0.05	0.14	0.74
A. taeniatus	Upper	0.19		0.22	0.02	0.57
	Middle	0.62	0.07	0.05	0.08	0.19
	Low	0.03	0.02	0.03	0.11	0.81

DISCUSSION

Food overlap between the two congeneric species was low in the least-disturbed region (upper Rio das Velhas), confirming our first hypothesis, that closely-related sympatric species diverge in their trophic niche to allow coexistence. In this study, the species *A. lacustris* and *A. taeniatus* presented high trophic plasticity in response to pollutants, increasing their food overlap and presenting similar isotopic signatures in the heavily polluted areas. Such aspect confirm our second hypothesis, that human disturbance promotes homogenization of fish species' diets. Despite plant and insect remnants were the predominant items in the stomachs of *A. lacustris* and *A. taeniatus*, algae and periphyton were also important food items (especially in lower sites). The importance of autochthonous resources as food items was highlighted in the partition analysis, which indicated that algae (in polluted regions) and periphyton (in least-disturbed region) were the most assimilated resources for both species.

The variation in $\delta^{13}C$ and $\delta^{15}N$ compositions and in stomach contents of *A. lacustris* and *A. taeniatus* along the Rio das Velhas highlight their generalistic habits and high trophic plasticity (Manna et al. 2012, Carvalho et al. 2015) probably as a result of resource availability found in the aquatic environment (Lobón-Cerviá and Bennemann 2000). The *A. taeniatus* also changes its $\delta^{15}N$ compositions between seasons (with enriched values in the dry season) which can be due changes on trophic levels (Vander Zanden et al. 1997), but also can occur in response to a higher enrichment in $\delta^{15}N$ values of resources in dry seasons.

The predominance of insects and plant remains in their stomach contents as well as the consumption of algae/periphyton are in agreement with the literature (e.g. Andrian et al. 2001, Casatti et al. 2003, Bennemann et al. 2005, Souza and Lima-Júnior 2013). It is likely that periphyton (in least-disturbed sites) and algae (in more degraded sites) are also being consumed indirectly through the consumption of insects. Castro et al. (2016) also observed a trend of changes in macroinvertebrates assimilation of algae and periphyton between degraded and preserved environments, which reinforces our statement. These changes in the type and proportion of autochthonous resources that sustain the two species are probably due to changes in environmental conditions as a result of pollution. Periphyton (or biofilm) is defined as an integral and independent micro-ecosystem in aquatic ecosystems, harboring biotic components (like algae, fungi, bacteria, protozoans, metazoans) and abiotic components (like substrata, extracellular polymeric substance and detritus) (Wu 2016). These organisms occur on the surface of rocks and submerged vegetation (Tundisi and Tundisi 2008), in environments with good water quality and greater presence of rocks and wood that favor the proliferation of periphyton. On the other hand, an increase of nutrients in the aquatic environment triggers a marked increase in algae (Tundisi and Tundisi 2008). Therefore, the expected greater abundance of algae in areas under the influence of pollution and of periphyton in sites with better environmental conditions, explain the shifts on basal resources assimilated by fish species.

In this study, stomach contents and stable isotopes analyses showed that there is a tendency to niche overlapping in *A. lacustris* and *A. taeniatus* in the presence of pollutants. The percentage of niche overlap observed by stomach contents and stable isotopes analyses were not the same, which is expected since not all items found in fish stomachs are assimilated (Manetta and Benedito-Cecílio 2003). In addition, the items consumed only occasionally or accidentally by individuals are observed on stomach contents, but will not be reflected on isotopic composition of fish. The greater overlap observed in the middle and lower course of Rio das Velhas could be due to the lower heterogeneity and resource availability in impacted sites (Gutiérrez-Cánovas et al. 2015). Fish tend to exhibit greater selectivity and specialization in the resources consumed in heterogeneous aquatic ecosystems, while in environments with few resources (or predominance of a single resource), fish tend to share the same food items (Knoppel 1970, Hurlbert 1978). Although we did not measure algae abundance, it is expected that in polluted sites populations reach high densities (e.g. Lata Dora et al. 2010, Macedo and Sipaúba-Tavares 2010), becoming an important food source consumed either directly or indirectly by generalist species, which can explain the higher niche overlap between *A. lacustris* and *A. taeniatus* in the middle and lower regions.

Trophic niche amplitude differed between regions. In the undisturbed region (Upper RV), both species had a broader trophic niche on the horizontal axis (niche with great carbon range). This trend is expected in food webs in which there are multiple basal resources with varying $\delta^{13}C$ values, enabling niche diversification at the base of a food web (Layman et al. 2007), which indicates that *A. lacustris* and *A. taeniatus* feed on a greater range of resources under natural conditions. On the other hand, in the most polluted region (Middle RV), the two species presented a narrow carbon range and a large nitrogen range (more vertical trophic niche). The narrow carbon range may be occurring in response to the restriction and homogenization of available food resources (the opposite of what has been observed in preserved sites). A larger range in $\delta^{15}N$ sometimes suggests more trophic levels and thus a greater degree of trophic diversity (Layman et al. 2007), however, probably this is not the explanation to the niche verticalization observed in this study, but the greater enrichment observed in the basal resources. This verticalization of the trophic niche has been found in fish (De Carvalho et al. 2017) and macroinvertebrates (Castro et al. 2016) in environments impacted by other anthropogenic activities (sugarcane).

The enriched nitrogen values of fish and resources especially in the middle section are probably related to the influence of sewage effluents, since $\delta^{15}N$ values of domestic wastes ranges between 7‰ to 38‰ (Dailer et al. 2010). Domestic wastes are nitrogen enriched especially because of isotopic fractionation during nitrification and volatilization in the case of ammonium, or denitrification in the case of nitrate (Nikolenko et al. 2018).

Therefore, the uptake of enriched $\delta^{15}N$ by primary producers are reflected in the entire food web (McClelland et al. 1997). Changes in $\delta^{15}N$ values along the pollution gradient, with a particularly large increase in regions affected by sewage effluent, were similar to those reported in studies of macroinvertebrates (e.g. Morrissey et al. 2013, Pastor et al. 2014, Baumgartner and Robinson 2016), and primary producers (e.g. MCclelland and Valiela 1998, Cole et al. 2004, Wang et al. 2016). Together, results of these studies, including ours, support the finding that high $\delta^{15}N$ values are good indicators of anthropogenic stress in aquatic systems.

Therefore, stomach contents and stable isotope analyses were very useful to evaluate the effects of the presence of pollutants in the trophic ecology of two congeneric species. It was possible to observe that even where species originally present different feeding habits (verified through the analysis of the stomach contents), food webs were mainly based on autochthonous items, such as algae and periphyton (verified through the isotopic analysis), assimilated directly and indirectly through aquatic insects. The presence of pollution, besides triggering increased food overlap between *A. lacustris* and *A. taeniatus*, also promoted an enrichment in $\delta^{15}N$ values of fish and resources. The $\delta^{15}N$ values of fish seems to be an effective means to detect anthropogenic impacts in aquatic ecosystems. In addition to providing important information on species biology, our work contributes to elucidate one of the 100 key ecological issues (Sutherland et al. 2013): How do resource pulses affect resource use and interactions between organisms?

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Supplementary material 1

Figure S1. Distribution of *A. lacustris* (red points) and *A. taeniatus* (blue points) species in the bi-plot space by study regions.

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